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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Pyrimidinone, muramyl dipeptide and tripeptide, detoxified lipopolysaccharide and trehalose dimycolate immunomodulators have been evaluated for ability to increase nonspecific resistance of CD-1 mice to Listeria monocytogenes, encephalomyocarditis virus and herpes simplex virus type 2. The brominated pyrimidinone was very effective in prophylactic treatment aginst the viral infections, but not against listeria infection by the regimens tested. Of the bacterial cell wall type immunomodulators, detoxified endotoxin and trehalose

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dimycolate appeared to be the most effective. Additional experiments to directly evaluate the same doses, regimens and vehicles are planned in normal CD-1 mice. The most effective agents will be tested for ability to enhance resistance in the face of ^{89}Sr-induced monocytopenia, granulocytopenia and depressed NK cell activity.

The mechanism of activation, and effects of the C. parvum immunomodulator have been investigated in ^{89}Sr treated or congenitally defective Sl/Sld mice; both provide models for deficient bone marrow dependent functions. Our studies in the ^{89}Sr system have established that C. parvum activation of peritoneal macrophages (MØ) is associated with a marked acute and persistent PMN inflammatory response. If this inflammatory response is decreased sufficiently by ^{89}Sr treatment, there is reduced MØ activation. Studies with C. parvum effects on MØ in ^{89}Sr treated and Sl/Sld mice have documented unexpected additional complexity among MØ populations. The data suggest at least three functionally definable populations on mononuclear phagocytes which appear to be independently regulated.

In the CD-1 mouse, we have extended our observations with C. parvum to several other biologic response modifiers known to activate MØ for antitumor activity (pyran copolymer and M. bovis strain BCG). BCG appears to activate peritoneal MØ relatively normally (as assessed by ectoenzyme phenotype and antitumor activity) whether the mice are able to mount a Mo-MØ rich peritoneal exudate or not. These data lend additional support to the concept that certain immunomodulators can activate resident MØ in situ without need for a Mo-MØ inflammatory influx. Additional studies have shown that ^{89}Sr treatment also does not appear to affect markedly the ability of resident, thiglycollate or C. parvum activated peritoneal MØ to proliferate in response to CSF. The two activated MØ populations had increased proliferative capacity, whether there was an inflammatory exudate of immature monocytes or not.

We have also begun deloping liposome methodology for depletion of tissue MØ. Studies in this year have been devoted to optimizing the lipsome constitution, and establishing the types of interaction of liposomes with MØ. Studies in the coming year with focus on the effects of toxins incorporated in liposomes on MØ function in vitro and in vivo.

PROGRESS REPORT - 1 July 1984 - 30 June 1985

1.0 Experimental Objectives

1.1 Continue to evaluate the efficacy of immunomodulators in our battery of microbial infections.

1.2 Define the effects of immunomodulators on various nonspecific effector cell populations in the 89Sr system.

1.3 Develop effective methods to decrease tissue M0 in normal and 89Sr treated mice.

2.0 Detailed Progress Report.

2.1 Evaluation of immunomodulators in a battery of microbial infections. The results reported in the 1 st Annual Report (August 1984) have been submitted for publication, and document: (i) CD-1 mice express relatively normal resistance to infection with EMC and HSV-2 viruses and to Listeria monocytogenes during the first 30 days after 89Sr treatment, despite profound monocytopenia, granulocytopenia and decreased NK cell activity; and (ii) a variety of immunomodulators, including C. parvum, avridine, and MVE-2 show relatively normal ability to enhance microbial resistance in 89Sr treated mice. During this year, studies have been performed in normal CD-1 mice with additional immunomodulators.

Progress was delayed during renovation of our animal facilities into an isolation area. We now have an excellent isolation system that should assure us of having clean animals. We believe that this is essential for meaningful experiments with immunomodulators. In last year's report we documented that inapparent infection with mouse hepatitis virus increased natural resistance of mice to subsequent infection with EMC virus. In collaboration with Dr. Abigail Smith at Yale, we have now documented that overt infection of mice with mouse hepatitis virus can activate peritoneal macrophages, as assessed by changes in ectoenyzme phenotype (Table 1).

During this year we have concentrated on testing two classes of immunomodulators: the pyrimidinones and bacterial cell wall derivatives or synthetic analogs. Single i.p. or oral prophylactic treatment of mice with 2-amino-5-bromo-6-phenyl-4-pyrimidinol (ABPP, Upjohn) was quite effective in increasing resistance to EMC, while i.p. or oral multiple dose prophylactic regimens were effective against HSV-2 infection (Table 2). There may be a [illegible] response to ABPP; in both infections, the higher dose systemic regimens of ABPP treatment appeared to be less effective than lower dose regimens. No gross drug toxicity was apparent, however. In contrast to the effectiveness against the viral infections, single or multiple dose prophylactic regimens of ABPP had little effect against listeria infection. No exacerbation of infection, however, was noted as has been observed with some other immunomodulaors. Future studies will be directed at oral treatment, and will assess prophylactic and/or therapeutic treatment regimens.

Sin[illegible] . or oral, prophylactic or therapeutic, treatment with

muramyl tripeptide-phosphatidylethanolamine (MTP-PE, Ciba-Geigy) was not markedly effective against either EMC or listeria infections (Table 3). Multiple dose, combined prophylactic/therapeutic treatment with MTP-PE was also ineffective against EMC virus, while a similar regimen with muramyl dipeptide (MDP, Choay Chimie Reactifs) was slightly effective. The more lipophilic derivative, MDP-glyceryldipalmitate (MDP-GDP, Choay) showed marginal activiy. Single prophylactic treatment with detoxified bacterial lipopolysaccharide (detox LPS, Ribi), trehalose dimycolate (TDM), or the combination, in a 2% squalene vehicle, was markedly effective against listeria infection, and somewhat effective against EMC virus (Table 3). More direct comparisons with these bacterial cell wall types of immunomodulators, with the same doses, vehicles and regimens, need to be performed before comparative efficacy can be established.

Dependent upon availability, other immunomodulators which we are particularly interested in testing for broad nonspecific antimicrobial activity include: gamma interferon, macrophage colony stimulating factor, IL-1, and several new synthetic materials.

2.2 Effects of immunomodulators in mice that have been selectively depleted of various effector cell populations.

2.21 Heterogeneity of MØ activation by C. parvum. The following section briefly summarizes results that are currently in press. In normal CBA/J mice, i.p. injection of C. parvum increases MØ colony forming precursors (MØ CFU) in the spleen, as well as increases the number of phagocytic MØ that bind IgG2a and IgG2b immune complexes. These phagocytic MØ suppress Con A induced lymphocyte proliferation, probably reflecting a 10-fold increase in prostaglandin E (PGE) in the splenic MØ. The 89Sr system was used to establish that these splenic suppressor MØ are derivatives of bone marrow MØ CFU. Treatment of mice with 89Sr reduced bone marrow MØ CFU to less than 1% of the normal levels for more than 30 days, and reduced circulating monocytes (Mo) to 5% by day 10 and 30% by day 30. Splenic MØ CFU increased 20-fold in 89Sr as compared with normal controls. However, C. parvum induced splenic suppressor MØ activity was sharply reduced in 89Sr treated mice despite the striking increase in splenic MØ CFU. The kinetics of recovery of splenic suppressor MØ showed a steady increase between 20 and 50 days after 89Sr, correlating with recovery of significant levels of bone marrow MØ CFU. These results suggest that radiosensitive bone marrow stem cells and/or the bone marrow microenvironment are necessary for the generation of both Mo and the splenic suppressor MØ, and that one stem cell might be common to both types of mononuclear phagocytes. This notion was explored further by employing congenitally anemic mice of the Sl/Sld genotype in which the hemopoietic microenvironment is genetically defective in supporting stem cell proliferation, differentiation and function.

The congenital defect in Sl/Sld mice was found to be additionally expressed by a monocytopenia of less than 10% of the levels in normal congenic littermate controls, and by failure of splenic MØ CFU to increase in response to C. parvum. PGE producing suppressor MØ, however, were induced normally by C. parvum in Sl/Sld mice. These data establish that signficiant impairment of the formation of Mo is part of the overall hemopoietic defect in Sl/Sld mice. The fact that PGE producing suppressor splenic MØ, however, were inducible in the presence of profound monocytopenia indicates that these suppressor MØ are independent of

mechanisms that regulate blood Mo production. The data in 89Sr mice showed the additional independence of the suppressor splenic MØ from splenic MØ CFU, because the latter increase above normal levels in 89Sr treated mice. Overall, these findings show a complexity among MØ populations that requires reclassification. At the simplest level, MØ can be classified as bone marrow dependent (Mo and splenic suppressor MØ) and independent (resident tissue MØ and splenic MØ CFU). The splenic suppressor MØ, however, are Mo independent although bone marrow dependent. The data suggest at least three functionally definable populations of mononuclear phagocytes which appear to be independently regulated.

2.22 Role of PMN in MØ activation by C. parvum.

Hadkill and his colleagues have hypothesized that phagocytosis of C. parvum by PMN is required for MØ activation by this immunomodulator. The 89 Sr system was used to render B6C3F1 and CD-1 mice granulocytopenic prior to i.p. injection of C. parvum. The subsequent granulocyte response in the peritoneal cavity was measured during both the acute (5 hr after C. parvum) and the chronic (7 day) inflammatory reactions. The 5 hr PMN influx was reduced by 85 and 86% in CD-1 mice given C. parvum 3 or 7 days respectively after 89Sr treatment. The 5 hr acute PMN influx in B6C3F1 mice was inhibited by 86% when C. parvum was administered 3 days after 89Sr, and 41% when C. parvum was administered at 7 days after 89Sr. The chronic, 7 day, PMN response was also depressed. PMN levels were reduced by 66-76% in CD-1 mice and by 83-89% in B6C3F1 mice. If an acute PMN influx is required for C. parvum complete activation of MØ, then MØ from 89Sr treated mice tested 7 days after C. parvum should possess lower alkaline phosphodiesterase I (APD-I) ectoenzyme activity indexes and lower cytotoxicity against tumor cells. We found that C. parvum MØ from normal or 89Sr treated mice showed normal activation as assessed by reduced 5'nucleotidase ectoenzyme activity (data not shown). C. parvum MØ from 89Sr treated mice in both mouse strains, however, exhibited markedly lower APD-I activity indexes than did activated MØ from normal mice (Table 4). C. parvum MØ from 89Sr treated B6C3F1 mice also exhibited markedly reduced cytotoxcity against the B16F10 melanoma tumor target cells (Figure 1). In these experiments, unfortunately, MØ from C. parvum activated normal CD-1 mice were not cytotoxic for B16F10 cells, so antitumor activity of MØ from the 89Sr mice could not be assessed. However, the APD-I activity indexes suggest that C. parvum MØ from 89Sr treated CD-1 mice would exhibit reduced antitumor activity against the B16F10 tumor target.

The present results differ somewhat from our previous data indicating completely normal activation of peritoneal MØ by C. parvum in 89Sr treated CD-1 mice (Infec. Immun., 1982; Lab. Inves. 1983). At present we have not completely established the reasons for the differences in results. However, the APD-I activity indexes in the previous work showed no difference between C. parvum activated MØ from 89Sr or control mice, correlating with their positive antitumor activity against Lewis lung carcinoma target cells. It is possible that the C. parvum preparations have changed; we, as well as other investigators, have noted increased chronic PMN exudation with C. parvum over the past few years. On the other hand, the differences in antitumor activity of C. parvum MØ from 89Sr treated mice may be related to the tumor target used (Lewis lung vs. B16F10); we have previously noted that Lewis lung cells are more sensitive to MØ antitumor activity than are B16 cells. Direct comparative experiments are planned to address these issues.

2.23 Effect of 89Sr on resident MØ and MØ activation by a variety of immunomodulators in CD-1 mice. We investigated the effect of 89Sr on MØ activation in CD-1 mice by C. parvum, thioglycollate broth (TG), pyran, and M. bovis BCG strain. In contrast to the results obtained with C. parvum, PMN and MØ levels in the peritoneal cavity after pyran (7 days) or BCG (14 days) administration were not reduced in the 89Sr treated CD-1 mice. Lymphocyte levels were reduced, however. BCG MØ from 89Sr treated mice exhibited the same ectoenzyme phenotype (reduced 5'N and APD activity) as did BCG MØ from normal mice, and both MØ populations were cytotoxic for Lewis lung carcinoma cells. With pyran MØ from normal or 89Sr treated mice, 5'N activity was markedly reduced in both; the APD-I and antitumor results were inconclusive. These results and those from the C. parvum studies point out that there may be significant differences among immunomodulators in mechanisms of MØ activation.

The effect of 89Sr on the ability of resident peritoneal MØ to proliferate in response to CSF, and the effect of TG and C. parvum were also investigated. Table 5 shows that treatment with 89Sr did not appear to affect markedly the proliferative capacity of Res Mϕ, nor the enhanced prolferative capacity of TG or CP MØ. These data in the CD-1 mouse, together with the MØ protein profiles, ectoenzyme phenotypes, antitumor activity, and intrinsic antiviral activity to HSV-1, indicate that at least in this mouse strain 89Sr treatment does not alter a number of resident MØ functions. The experimental results also support the concept that certain immunomodulators can activate the Res peritoneal MØ in CD-1 mice without a necessary influx of inflammatory monocytes.

2.24 Effect of specific activity of 89Sr, route of administration, and mouse strain on cellular depletion. The CD-1 mouse appears to be particularly sensitive to 89Sr, in that circulating Mo, PMN, and NK cells are profoundly suppressed within a week after treatment with 89Sr of either high (6000 Ci/g) or low (100 mCi/g) specific activity. Data with the B6C3F1 mouse have emphasized the importance of mouse strain in cellular suppression; NK cell activity in the B6C3F1 mouse remains high for at least one month after treatment with either high or low SA 89Sr (data not shown). Other experiments in CBA/J mice have shown a close dependence of both the magnitude and duration of suppression of circulating Mo and bone marrow MØ CFU with the specific activity of 89Sr. In contrast to these variations with mouse strain and SA of 89Sr, there are insignificant differences between 89Sr administered i.v. or i.p to CD-1 or B6C3F1 mice (data not shown).

2.3 Development of liposome methodology to decrease tissue MØ in normal and 89Sr treated mice.

2.31 Liposome preparation. Liposomes have been prepared by the method of solvent exchange starting with lipids dissolved in chloroform, then evaporated to dryness under a stream of nitrogen. The lipids are then dispersed in saline and dispersed to form liposomes in an ultrasonic field. Preparations have included liposomes prepared from phosphatidylcholine (PC), phasphatidic acid (PA), phosphatidyl serine (PS), cholesterol and iodocholesterol. The reference preparation is PC:PS:cholesterol in a 7:1:3 molar ratio. This has yielded a negatively charged multilamellar liposome of 200-600 A as estimated by electron microscopy. We employed two markers to quantitate liposome uptake by

MØ: iodocholesterol which is quantitated by x-ray fluorospectrometry (courtesy of Dr. John Bray, ECU School of Medicine), and 3H-cholesterol which is quantitated by liquid scintillation counting.

2.32 Liposome uptake. Studies have employed two sources of MØ for determining optimum conditions for liposomal uptake: mouse resident peritoneal MØ and the P388D1 continuous mouse MØ cell line.

2.321 Effect of chemical consitituion. Constitution of liposomes with PA and PS results in a negative charge on the vesicles; deletion of PA and PS results in a neutral vesicle. Uptake of negatively charged liposomes by P388D1 and peritoneal MØ was significantly greater than of neutral liposomes. The uptake of negatively charged liposomes by P388D1 cells varied inversely with the cholesterol content of the liposomes. The levels of cholesterol used as carrier in the 3H-cholesterol studies, however, were too low to affect the results negatively. Uptake of liposomes constituted with equimolar amounts of either PA or PS phospholipids was comparable.

2.322 Uptake by suspended or adherent MØ. MØ (2 x 10 in 1 ml Ca and Mg free PBS containing 0.2 ml liposome suspension) were tumbled for varying times at 37C. After washing twice, the cells were pelleted and counted. Similar numbers of MØ adherent to 35mm petri dishes after 24 hr were washed and liposomes (0.2 ml diluted to 1 ml in PBS) were added. After varying intervals the cells were lysed with NP40, the lysate washed out with PBS and counted. Cell associated radioactivity was ten-fold higher in the suspension preparations than among adherent cells, suggesting a higher efficiency of the liposome-MØ contact events.

2.323 Effect of temperature on uptake. One serious question is whether cell-associated activity of marked liposomes signifies binding to the cell membrane, endocytosis or both. Reduction of temperature to 4 C essentially halts endocytosis. Parallel preparations of P388D1 cells in suspension and adherent to petri dishes were incubated for increasing intervals from 5-180 min. The steepest increase in radioactivity was found to take place in the first 15-30 min. Cell associated activity increased linearly at lower rates at 37C from these points to the last sampling at 180 min. At 4 C the increase among suspensions was 50% less than at 37C (Fig. 1). In the adherent cells at 4C, no progressive increase was seen after 15 min. The data suggest that a substantial amount of cell associated radioactiviy represents liposomes on the cell surface and not within the cell. This could be an important consideration in preparing liposomes with which to transport agents into cells. We are currently planning to test the effects of liposomes containing the ricin A chain on MØ in vitro and in vivok. The reults of these experimets should provide additional information concerning liposome uptake and processing in MØ.

3.0 Publications/presentations related to this project.

3.1 Manuscripts

Sh bata, Y. and A. Volkman. Ontogeny and differentiation of mononuclear phagocytes. I. The effect of bone marrow depletion on prostaglandin E producing suppressor macrophages in mouse spleen. J. Immunol., in press 1985.

Shibata, Y. and A. Volkman. Ontogeny and differentiation of mononuclear phagocytes. II. The effect of hemopoietic microenvironment on suppressor macrophages in the congenitally anemic mice of the

Sl/Sld. J. Immunol., in press 1985.

Shibata, Y., W.L. Dempsey, P.S. Morahan and A. Volkman. Selectively eliminated blood monocytes and splenic suppressor macrophages in mice depleted of bone marrow by strontium 89. J. Leuk. Biol., in press 1985.

Morahan, P.S., A. Volkman, W.L. Dempsey, and J. Connor. Antimicrobial activity of various immunodulators: independence from normal levels of circulating monocytes and NK cells. Submitted.

Dempsey, W.L., A. Smith, A. Volkman and P.S. Morahan. Effect of inapparent murine hepatitis virus infection on mononuclear phagocytes and resistance to another virus infection. In preparation.

3.2 Abstracts and presentations

Ackermann, M.F., W.L. Dempsey, P. Hwu, D. Tenney, E. Leake and P.S. Morahan. Effect of PMN depletion on MΦ activation by P. acnes. Eastern Pennsylvania Branch of the American Society of Microbiology, February 1985.

Tenney, D.J., M.F. Sit, E.R. Leake, H.T. Largen and P.S. Morahan. Effect of 89Sr on protein synthesis and intrinsic resistance of peritoneal macrohages to HSV-1. Reticuloendothelial Society annual meeting, August 1985.

4.0 Research Plan

4.1 Continue to evaluate immunomodulators for antimicrobial efficacy in normal CD-1 mice and in mice with selective defects in various effector cell populations. We will complete our studies with the pyrimidinone, MDP-PE, MTP, detoxified endotoxin, and trehalose dimycolate, and continue efforts to obtain other potentially interesting compounds to test. Depending upon the results, one or more of these compounds will be selected for test in 89Sr treated mice.

4.2 Define the effects of selected immunomodulators on effector cell populations in mice depleted of various cells. The studies with C. parvum as a prototype will be continued in order to determine the contributions of ontogenetic and environmental factors to MΦ activation, by infusing lethally irradiated mice with spleen from 89Sr treated syngeneic donors. As interesting compounds are identified in the host resistance studies, the mechanisms of immunomodulatory activity of these may also be explored in the 89Sr or Sl/Sld mouse systems. However, we expect that most of our efforts will be focused on Obectives 1 and 3 during our final contract period.

4.3 Develop effective methods to decrease tissue MΦ in normal and 89Sr treated mice. Experiments will be focused on in vivo approaches, particularly the delivery of ricin (and possibly other toxic agents) and later, astatine-211, in normal and 89Sr treated mice. We will study the fate of the liposome inoculum, and the consequences of liposome-toxin injection on resident MΦ populations at the site and remote from injection in regard to cell survival and function. We will also assess effects on host resistance to infection. Effects on non-MΦ populations such as NK cells, PMN and lymphocytes may also be investigated. Finally, the effects of liposome-toxin treatment on the capacity for cell recovery and modulation by selected biologic response modifiers will be addressed.

TABLE 1

Effect of Mouse Hepatitis Virus (MHV) Infection of Peritoneal MØ Ectoenzyme Levels[a]

Day after infection	5'N	APD
3	19.8 ± 8.8	14.1 ± 0.8 [b]
5A	14.2 ± 4.8	10.9 ± 1.5 [b]
B	12.4 ± 2.4 [b]	9.1 ± 0.6 [b]
7-8	10.9 ± 1.2 [b]	20.8 ± 1.4
10	1.1 ± 1.0 [b]	18.0 ± 1.5
Control 1[c]	19.5 ± 3.5	22.8 ± 0.2
Control 2[c]	24.2 ± 2.8	18.1 ± 2.8

[a] Balb/c mice were infected with the JHM strain of murine hepatitis virus.

[b] Represents a significant reduction ($p < 0.05$) in activity as compared with the combined controls.

[c] Controls were taken at the beginning (1) and the end (2) of the study.

TABLE 2

Protective Effects of ABPP Immunomodulator Against Infections in Normal CD-1 Mice

Drug	Treatment: Dose (mg/kg)	Route	Regimen (day)	EMC: Mortality Dead/Total	EMC: Mortality %	EMC: Survival Median Day	Listeria monocytogenes: Mortality Dead/Total	Listeria monocytogenes: Mortality %	Listeria monocytogenes: Survival Median Day	HSV-2: Mortality Dead/Total	HSV-2: Mortality %	HSV-2: Survival Median Day
CMC	1.0%	I.P.	-1 or -7	14/15	(93%)	4.4	15/15	(100%)	3.0	14/15	(93%)	10.8
MVE-2	50	I.P.	-1	3/10	(30%)*	>14.0*	ND			0/10	(0%)*	>21.0*
C.parvum	35	I.P.	-7	ND			0/10	(0%)*	>14.0*	ND		
ABPP	200	I.P.	-7	6/10	(60%)*	8.3*	7/10	(70%)*	6.5	ND		
ABPP	200	I.P.	-1	3/10	(30%)*	>14.0*	10/10	(100%)	3.3	ND		
ABPP	100	I.P.	-1	1/10	(10%)*	>14.0*	ND			ND		
ABPP	200	P.OS	-1	2/10	(20%)*	>14.0*	ND			ND		
ABPP	100	P.OS	-1	7/10	(70%)	7.1	ND			ND		
ABPP	200	I.P.	-2,-1,-1/3	ND			10/10	(100%)	2.5	ND		
ABPP	200	P.OS	-2,-1,-1/3	ND			9/10	(90%)	5.6	ND		
ABPP	200	I.P.	-3,-2,-1	ND			ND			9/10	(90%)	11.1
ABPP	200	P.OS	-3,-2,-1	ND			ND			3/9	(33%)*	16.8*
ABPP	100	I.P.	-3,-2,-1	ND			ND			4/10	(40%)*	17.1*
ABPP	100	P.OS	-3,-2,-1	ND			ND			5/10	(50%)	15.0

* $p < 0.05$ as compared with placebo control, using the Chi Square test for mortality data and Mann Whitney U test for survival analysis.

TABLE 3

Protective Effects of Bacterial Cell Wall Types of Immunomodulators Against Infections in Normal CD-1 Mice

				EMC			Listeria monocytogenes		
	Treatment			Mortality		Survival	Mortality		Survival
Drug	Dose (mg/kg)	Route	Regimen (Day)	Dead/ Total	(%)	Median Day	Dead/ Total	(%)	Median Day
CMC	1.0%	I.P.	-1 or -7	14/15	(93%)	4.1	15/15	(100%)	3.5
MVE-2	50	I.P.	-1	0/10	(0%)*	10.0*	ND		
C.parvum	35	I.P.	-7	ND			1/10	(10%)*	9.3*
MTP-PE	1	I.P.	-7	9/10	(90%)	4.7	10/10	(100%)	4.0
MTP-PE	1	P.OS	-7	10/10	(100%)	4.2	8/10	(80%)	5.6
MTP-PE	1	I.P.	-1	9/10	(90%)	5.0	ND		
MTP-PE	1	P.OS	-1	10/10	(100%)	4.4	ND		
MTP-PE	1	I.P.	+2	ND			8/10	(80%)	5.2
MTP-PE	1	P.OS	+2	ND			7/10	(70%)	6.3
NaCl	0.85%	I.P.	-1,0,+1,+2	13/15	(87%)	5.6	ND		
MDP	4	I.P.	-1,0,+1,+2	4/10	(40%)*	8.6	ND		
MDP	1	I.P.	-1,0,+1,+2	9/10	(90%)	5.0	ND		
MDP-GDP	4	I.P.	-1,0,+1,+2	9/10	(90%)	4.7	ND		
MDP-GDP	1	I.P.	-1,0,+1,+2	5/9	(56%)	7.6	ND		
CMC	1.0%	I.P.	-1,0,+1,+2	5/10	(50%)	8.1	ND		
MTP-PE	1	I.P.	-1,0,+1,+2	10/10	(100%)*	3.9*	ND		
CMC	1.0%	P.OS	-1,0,+1,+2	10/11	(91%)	4.9	ND		
MTP-PE	1	P.OS	-1,0,+1,+2	9/10	(90%)	5.0	ND		
Squalene	2%	I.P.	-7	10/10	(100%)	3.7	8/10	(80%)	8.2
Detox LPS	4	I.P.	-7	7/10	(70%)	6.6	0/10	(0%)*	>12.0*
TDM	4	I.P.	-7	7/10	(70%)*	6.4	0/10	(0%)*	>12.0*
Detox LPS +TDM	4+4	I.P.	-7	5/10	(50%)*	8.3*	0/10	(0%)*	>12.0*

* $p < 0.05$ as compared with placebo control, using the Chi Square test for mortality data and Mann Whitney U test for survival analysis.

TABLE 4

Effect of ^{89}Sr on APD-I activity index[a]

	B6C3F1 Mice		CD-1 Mice	
	Exp. 1[b]	Exp. 2[c]	Exp. 1	Exp. 2
Control	10.0	10.0	29.2	33.7
^{89}Sr	3.9[b]	3.0[c]	5.9[b]	5.56[c]

[a]Ratio of the APD specific activity (SA) between untreated resident MØ and the APD S.A. of MØ activated with C. parvum.

[b]APD-I activity index in mice injected with C. parvum 3-4 days after ^{89}Sr.

[c]APD-I activity index in mice injected with C. parvum 7-8 days after ^{89}Sr.

TABLE 5

Effect of ^{89}Sr on MØ CFU in the Peritoneal Population

	^{89}Sr SA	MØ CFU / 10^3 MØ Plated					
	(m Ci/gr Sr)	Res	+^{89}Sr	C. parvum	+^{89}Sr	TG	+^{89}Sr
Exp 1	6,000,000	3.2	4.0	16.6	35.0	12.6	14.3
Exp 2	100	5.1	1.5	75.8	49.1	ND	102.2

Peritoneal cells were plated in liquid medium containing 10% FCS, 10% HoS, 10% LCM in a MEM and incubated for 2 weeks (Exp 1) or 3 weeks (Exp 2). Cultures were stained with Dif-Quik, and colonies containing more than 50 cells were counted.

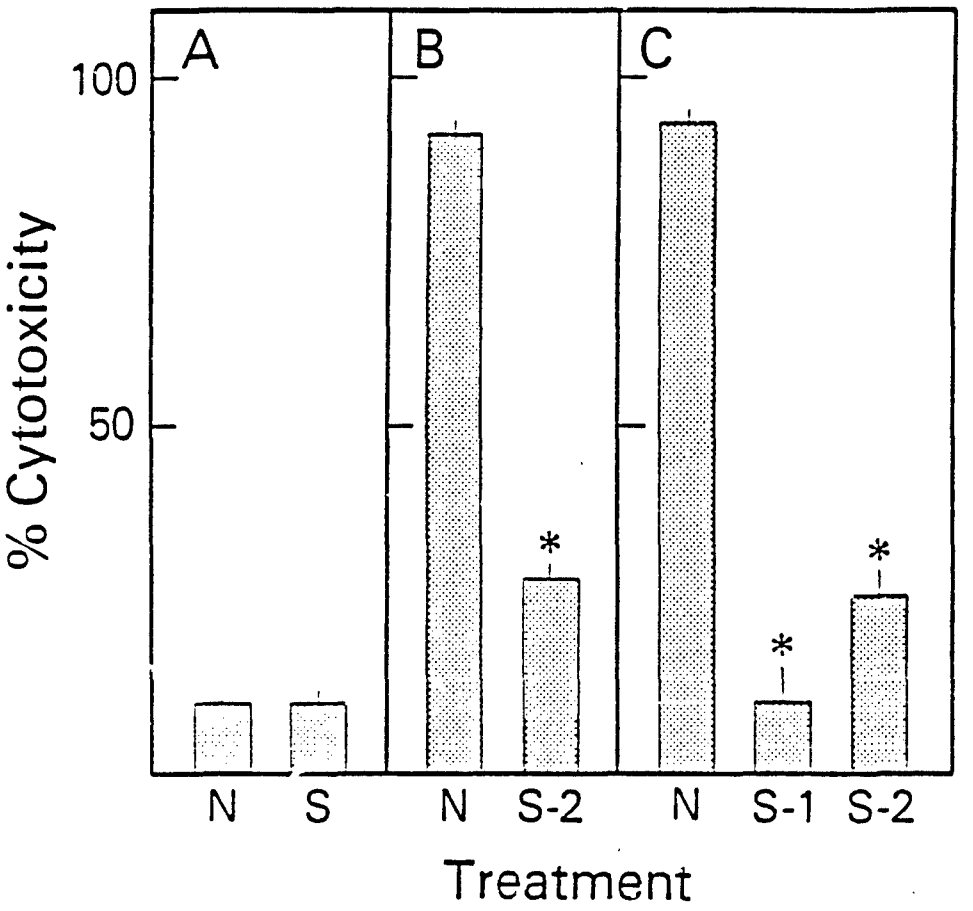

Figure 1 : Effect of ^{89}Sr on the AT activity against B16F10 melanoma target cells of resident (A) and activated MO four (B) and seven (C) days after P.acnes administration The MO:target ratio is 10:1. Data are expressed as the me (SEM) from four different experiments with 5-8 animals per treatment group processed individually.

N = Untreated (A) or P.acnes activated (B,C) MO from control mice.

S = Resident MO from mice treated with ^{89}Sr.

S-1 = P.acnes injected 3 or 4 days after ^{89}Sr treatment.

S-2 = P.acnes injected 7 or 8 days after ^{89}Sr treatment.

* = $P < 0.05$ as compared with P.acnes activated MO fror naive control mice.

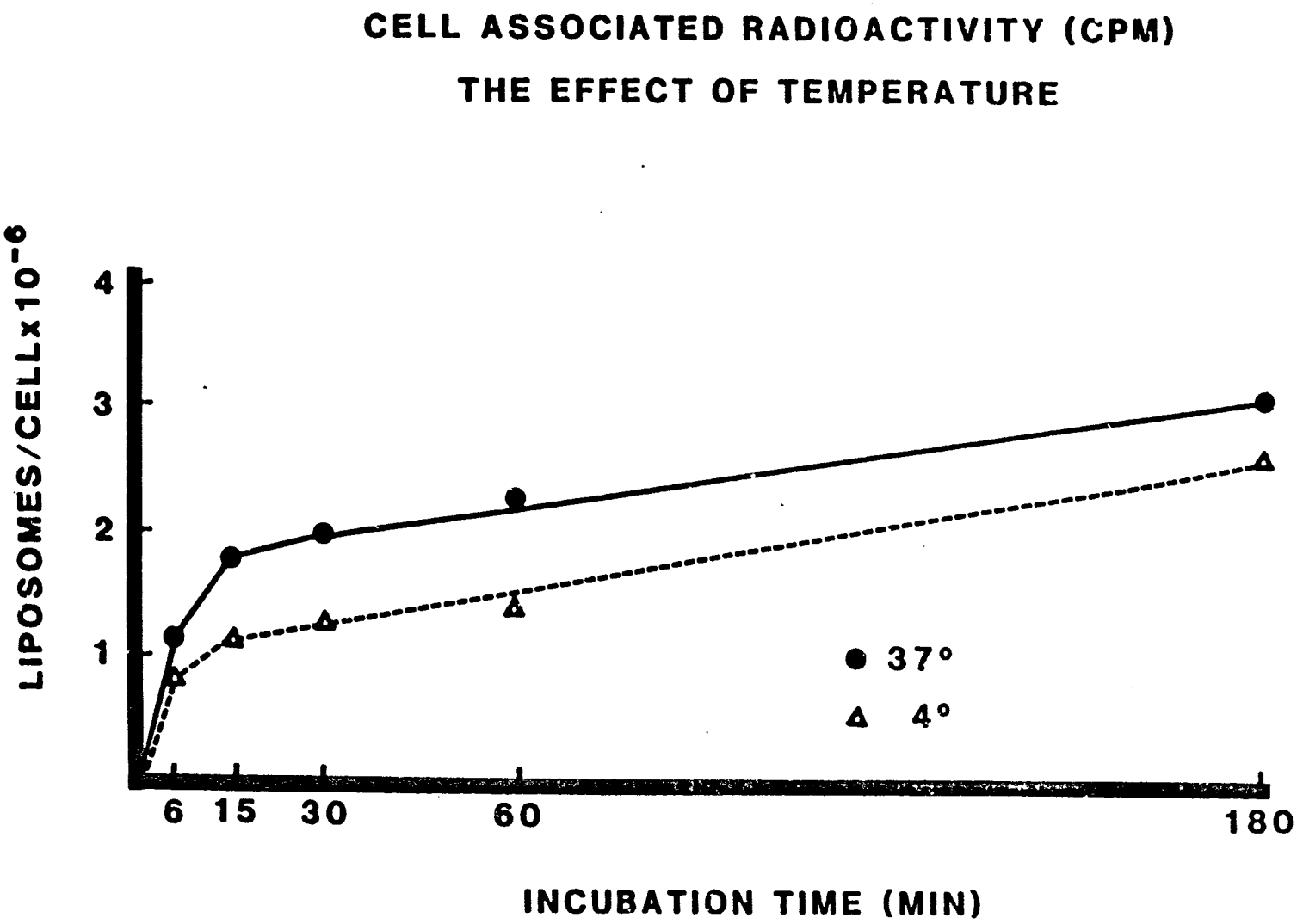
CELL ASSOCIATED RADIOACTIVITY (CPM)
THE EFFECT OF TEMPERATURE
LIPOSOMES/CELLx10⁻⁶
4
3
2
1
37°
4°
6
15
30
60
180
INCUBATION TIME (MIN)

Fig 2

END

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